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Effects of albendazole on the clinical outcome and immunological responses in helminth co-infected tuberculosis patients: a double blind randomised clinical trial

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ABSTRACT

Despite several review papers and experimental studies concerning the impact of chronic helminth infection on tuberculosis in recent years, there is a scarcity of data from clinical field studies in highly endemic areas for these diseases. We believe this is the first randomised clinical trial investigating the impact of albendazole treatment on the clinical and immunological outcomes of helminth co-infected tuberculosis patients. A randomised, double-blind, placebo-controlled trial of albendazole (400 mg per day for 3 days) in helminth-positive tuberculosis patients was conducted in Gondar, Ethiopia. The primary outcome was clinical improvement (ΔTB score) after 2 months. Among secondary outcomes were changes in the levels of eosinophils, CD4+ T cells, regulatory T cells, IFN-γ, IL-5 and IL-10 after 3 months. A total of 140 helminth co-infected tuberculosis patients were included with an HIV co-infection rate of 22.8%. There was no significant effect on the primary outcome (ΔTB score: 5.6 ± 2.9 for albendazole versus 5.9 ± 2.5 for placebo, $P = 0.59$). The albendazole-treated group showed a decline in eosinophil cells ($P = 0.001$) and IL-10 ($P = 0.017$) after 3 months. In an exploratory analysis after 12 weeks, the albendazole treated group showed a trend towards weight gain compared with the placebo group (11.2 ± 8.5 kg versus 8.2 ± 8.7 kg, $P = 0.08$)). The reductions in eosinophil counts and IL-10 show that asymptomatic helminth infection significantly affects host immunity during tuberculosis and can be effectively reversed by albendazole treatment. The clinical effects of helminth infection on chronic infectious diseases such as tuberculosis merit further characterisation.

1. Introduction

Similar to tuberculosis (TB), it has been estimated that one-third of the global human population is infected with intestinal parasites (World Health Organization (WHO), 2012; Salgame et al., 2013). Several studies, including some from Ethiopia, have shown an increased rate of helminth infection in TB patients compared with household contacts living in the same room with active TB patients and the healthy population (Tristão-Sá et al., 2002; Abate et al., 2012). The impact of helminth infection on the risk of developing TB and during the course of active TB is not well understood but is believed to involve helminth-induced effects on cell mediated immunity (Bentwich et al., 1999; Borkow et al., 2001; Elias et al., 2006).

Protective immunity in TB has been shown to be dependent on T-helper 1 (Th1) CD4+ T cells producing IFN-γ and TNF-α, as well as cytolytic T cells (CTLs) producing granule-associated cytolytic effector molecules (Brighenti and Andersson, 2012). It has been postulated that helminth co-infection could modulate the protective Th1-response against TB through an effect on the Th1/Th2 balance, with increased Th2 dominance and activity of regulatory

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T cells (Tregs) (Bentwich et al., 1999; Borkow et al., 2001). Such Th2 and Treg responses induce the production of cytokines including IL-4 and IL-10 which are potent inhibitors of the Th1 response (Rook, 2007; Redford et al., 2011). Indeed, in both humans and mice there is a marked increase in Th2-related cytokines such as IL-4, IL-5 and IL-13 following helminth infection (Anthony et al., 2007). In human volunteers, hookworm infection is associated with strong local and systemic Th2 and Treg responses (Gaze et al., 2012). The vaccine currently used against TB, Bacillus Calmette Guerin (BCG), is not effective against adult pulmonary TB in sub-Saharan Africa (Tameris et al., 2013). One reason could be that helminth infection modulates the immune response by skewing the Th1/Th2 balance and thereby attenuating the effects of the BCG vaccine (Elias et al., 2008). Several experimental studies confirm the immunomodulatory effect of helminths during TB (Bentwich et al., 1999; Borkow et al., 2001; Elias et al., 2005; Potian et al., 2011; Salgame et al., 2013). One of these studies clearly showed that *Nippostrongylus brasiliensis* infection was associated with impaired killing of *Mycobacterium tuberculosis* (Mtb), an effect primarily mediated by a switch to alternatively activated macrophages (AAMs) by IL-4 (Potian et al., 2011).

Among the very limited human studies on the interaction between helminths and active TB, one study showed lower IFN-γ and enhanced IL-10 responses (Resende Co et al., 2007). In our recent study from Ethiopia we found that asymptomatic helminth infection in TB patients showed effects on host immunity in terms of increased IgE and eosinophil levels (Abate et al., 2012). However, clinical studies investigating the impact of deworming during active TB in humans remains very limited. Thus, our aim was to investigate the clinical and immunological impacts of helminth infection in a randomised clinical trial.

2. Materials and methods

2.1. Study participants

Following written informed consent, newly diagnosed patients with pulmonary TB presenting consecutively from 1 March 2009 to 15 October 2012 at the Directly Observed Treatment Short-Course (DOTS) Clinics of the Teaching and Referral Hospital of the University of Gondar, the Gondar Health Centre and at Debark Hospital, Ethiopia were eligible for enrolment. After enrollment, helminth co-infected TB patients were randomly allocated, after 2 weeks of anti-TB treatment, to albendazole treatment (400 mg/day for three consecutive days) or identical placebo tablets. All helminth-positive TB patients, including the placebo group, received anti-helminth treatment at week 12 when the follow-up was completed. The TB treatment consisted of isoniazid, rifampicin, ethambutol and pyrazinamide for the first 2 months followed by 4 months of isoniazid and rifampicin. The inclusion criteria were that patients were 15–60 years old, with a positive smear result for acid-fast bacilli (AFB; smear-positive TB) or with clinical and chest X-ray (CXR) results suggestive of pulmonary TB (smear-negative TB) according to the WHO-based national guidelines (WHO, 2012). The exclusion criteria were that patients required hospital admission, were pregnant, infected with *Schistosoma* spp., displayed symptoms of active helminth infection such as diarrhoea or stomach cramps, or clinical signs or medical treatment indicating any concomitant chronic or infectious disease other than TB/HIV.

The study received ethical clearance from the Ethics Review Board of the University of Gondar, Ethiopia and from the Medical Ethics Board at Linköping University, Sweden. In addition, approval was obtained from the federal Drug Administrative and Control Authority (DACA), Ethiopia. The data collection and patient follow-up were monitored by an independent data and safety monitoring board (DSMB).

2.2. Study outcome

The primary outcome was a TB score change (ΔTB score) at week 8 compared with baseline. The secondary outcomes were sputum smear conversion after 2 months, changes in the CXR pattern from baseline to week 12, CD4+ T cell counts, IgE and eosinophil responses, as well as changes in the frequency of Tregs and IFN-γ, IL-5 and IL-10 producing peripheral blood mononuclear cells (PBMCs) after 3 months.

2.3. Randomization

Random numbers were generated in a block size of eight by the Addis Continental Institute of Public Health, Ethiopia. The treatment allocated for each patient was concealed in an individual envelope. Albendazole and placebo tablets were produced by the Addis pharmaceutical company, Ethiopia which is accredited for Good Clinical Practice (GCP). All tablets looked identical and were assigned a treatment code by the manufacturer. Both the investigators and clinic staff were blinded to the treatment given. The treatment code was kept in a sealed envelope at the manufacturer and opened after the last patient had been to a follow-up visit and the data had been analysed.

2.4. Clinical and laboratory analyses

2.4.1. Socio-demographic characteristics and assessment of the TB score

A structured questionnaire was used to collect socio-demographic and clinical information. As previously described (Wejse et al., 2008; Janols et al., 2012), a clinical score (TB score) which ranges from 0 to 13 points was assessed at baseline and 2 months after anti-TB treatment. The score is composed of signs and symptoms of TB each contributing one point (cough, haemoptysis, chest pain, dyspnoea, night sweating, anaemic conjunctivae, lung auscultation finding, tachycardia ($\geqslant$100/min), temperature ($\geqslant$37 °C), body mass index (BMI) $\leqslant$18 kg/m^2, BMI $\leqslant$16 kg/m^2, mid-upper arm circumference (MUAC) $\leqslant$220 mm, and MUAC $\leqslant$200 mm). As previously described (Wejse et al., 2008), the TB score was divided into three severity classes (SC-I-III) at baseline where SC-I was 0–5 points, SC II 6–7 points and SC-III 8–13 points.

2.4.2. CXR evaluation

Grading of CXR findings of pulmonary TB was done according to the National Tuberculosis Association of the USA as normal, minimal, moderately advanced and far advanced TB (American Thoracic Society, 1961). For statistical evaluation, this grading was translated to a semi-quantitative scale, 0 (normal), 1 (mild), 2 (moderate) and 3 (far advanced TB). During follow-up at 3 months, the initial CXR was used for comparison and the radiological response was classified according to a semi-quantitative scale, (1: normalised, 2: regression, 3: no change, 4: progress). The CXRs were read by the same senior radiologist and reading was blinded for HIV status and treatment assignment.

2.4.3. HIV screening and determination of CD4+ T cell count

Testing for HIV was done at the voluntary counseling and testing clinics, and at the DOTS clinic as part of the clinical routine with HIV rapid test kits (Shanghai Kehua Bio-engineering Co., Ltd. (KHB HIV 1/2 rapid test strip) China), Stat-Pak (HIV 1/2, Chembio Diagnostics Inc., USA) and Unigold (Trinity Biotech, USA). HIV-positive patients were referred to the HIV clinics for further assessment and free antiretroviral treatment (ART) according to the Ethiopian HIV/AIDS

treatment guideline (Federal Ministry of Health, HIV/AIDS Prevention and Control Office, Drug Administration and Control Authority, 2003). The ART consisted of a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI), according to the national Ethiopian HIV guideline. The $CD4^+$ T cell count was analysed using FACSCount (Becton Dickinson, San Jose, California, USA).

2.4.4. Determination of IgE and eosinophil counts

Serum IgE was determined with a commercial ELISA kit (Immundiagnostik, Germany) according to the manufacturer's instructions. The absolute eosinophil count of peripheral blood was computed in cells/mm^3 from the value of total and differential white blood cell counts obtained using Cell Dyn 1800 (Abbot, USA).

2.4.5. Stool examinations

Stool samples were collected on three consecutive days from each participant and examined using direct microscopy and Kato-Katz techniques (Sleigh et al., 1982) by the same technician throughout the study. The classification into helminth-positive or -negative was based on the examination of all three samples together from each patient. One in 10 slides were randomly selected and checked again by a second microscopist for quality control. The same stool sample collection and examination strategy was used at week 12.

2.4.6. Sputum smear examinations

AFB staining and examination were done on morning sputum samples at baseline and week 8 for three consecutive days. Sputum conversion was defined as three consecutive sputum smears becoming negative for AFB at week 8.

2.4.7. Isolation of PBMCs from whole blood

PBMCs were isolated by the density gradient separation method using lymphoprep solution as described previously (Lyke et al., 2005) and stored at −80 °C in FCS containing 10% DMSO. Trypan-blue exclusion dye (Sigma–Aldrich, USA) was used for detection of cell viability and only cells from patients with viability above 75% were used for experimental assays.

2.4.8. Analysis of Tregs

PBMCs were stained with fluorochrome-labeled monoclonal mouse anti-human antibodies CD4-FITC (BD Biosciences), CD25-Percp Cy5.5 (BD Biosciences), and CD127-Alexa 647 (BD Biosciences), followed by fixation/permeabilisation using cytofix/cytoperm solution (BD Biosciences) and intracellular staining with monoclonal antibodies (mAbs) against Foxp3− phycoerythrin (PE) (BD Biosciences). Cells were gated first, based on forward and side scatter, to exclude dead cells and cell debris. Fluorescence Minus One (FMO) controls were used for gating purposes. Tregs were defined as the population of cells that were CD4+/CD25hi/CD127low/FOXP3+. Flow cytometry data were collected on a FACSCalibur (BD Biosciences) using CellQuest acquisition software and were then analysed using Flowjo 7.6.5 (Tree Star).

2.4.9. Analysis of IFN-γ, IL-5 and IL-10 by ELISPOT

PBMCs (250,000/well) were plated onto ester-cellulose-bottomed plates (PVDF plates, Mabtech, Sweden) coated with a capture mAb specific for human IFN-γ (1-D1K), IL-5 (TRFK5) and IL-10 (9D7) at 15 μg/ml in PBS. Cells were incubated in a humidified incubator at 37 °C in 5% CO_2 for 24 h with purified protein derivate (PPD) antigens obtained from Mtb in duplicate using unstimulated and anti-CD3-stimulated cells as negative and positive controls, respectively. Spots were developed following the manufacturer's instructions (Marin et al., 2010).

2.5. Statistical analysis

Analysis was by intention to treat. Continuous data are expressed as means with S.D. Effects of deworming compared with placebo on primary and secondary outcomes were evaluated by chi-square test or Fisheŕs exact test for discrete variables and Student's *t*-test for continuous variables using STATISTICA software (Tulsa, USA). The percentage of relative change (PRC) (before and after deworming) was calculated as follows: $PRC = [(X_2 - X_1)/X_1] \times 100$ (Borkow and Bentwich, 2004). Multiple regression analysis was performed for primary and secondary outcome variables with a P value <0.1 in a univariate analysis including age, sex and HIV in the model. $P < 0.05$ was regarded as statistically significant.

In our previous study (Janols et al., 2012), newly diagnosed TB patients showed a 75% decline from their baseline TB score after 2 months of anti-TB treatment only. As no previous study on the effect of deworming on the clinical outcome had been conducted, the total sample size without considering sub-group analyses was a crude estimation calculated under the assumption that 210 helminth-positive TB patients would have to be included, giving 80% power at the 5% significance level, to detect a difference in treatment response of 15% including a 5% drop-out rate.

3. Results

3.1. Study population

A total of 1251 newly diagnosed pulmonary TB patients were screened for eligibility and of those, 846 TB patients were excluded (Fig. 1). Of the remaining 405 eligible TB patients, 265 were helminth-negative and were excluded from participation in the randomised trial. A total of 140 helminth-positive TB patients were randomly allocated to albendazole ($n = 72$) or placebo ($n = 68$) treatments. No adverse reaction or adverse event was reported due to albendazole treatment.

3.2. Baseline characteristics

The rate of HIV co-infection was 22.8% (32/140). No significant difference in HIV co-infection rate was observed between the albendazole (25%) and the placebo (21%) groups (Table 1). From the total HIV-positive TB patients assigned to the albendazole group, 94% were on ART while all HIV-positive TB patients in the placebo group were on ART. There were no significant baseline differences between the albendazole and the placebo groups in the proportion of patients in the three severity classes of the TB score where 63% versus 52% were in SC-1 ($P = 0.19$), 19% versus 29% were in SC-II ($P = 0.17$) and 18% versus 19% were in SC-III ($P = 0.87$) without any sub-group differences with regard to HIV. Furthermore, no differences were detected in the baseline TB score between the albendazole and placebo groups, including after sub-grouping for HIV (7.8 ± 2.5 versus 7.5 ± 2.7 points, $P = 0.53$ for HIV-negative and 8.8 ± 2.4 versus 8.7 ± 2.2 points, $P = 0.93$ for HIV-positive patients in the albendazole and placebo groups, respectively). At week 2, this pattern was stable (3.2 ± 1.9 versus 3.3 ± 1.9, $P = 0.69$) in the albendazole and placebo groups, respectively, without any differences with regard to HIV status.

At baseline, a significantly lower CD4+ T cell count in the albendazole group compared with placebo (489 ± 227 cells/mm^3 versus 642 ± 328 cells/mm^3; $P = 0.025$, Table 1) was found mainly in the HIV-negative patients whereas no such baseline difference was detected between the treatment groups among HIV-infected patients (168 ± 119 cells/mm^3 versus 190 ± 129; $P = 0.62$). *Ascaris lumbricoides* was the most common helminth species at a prevalence of 52% in TB patients. There were no significant differences

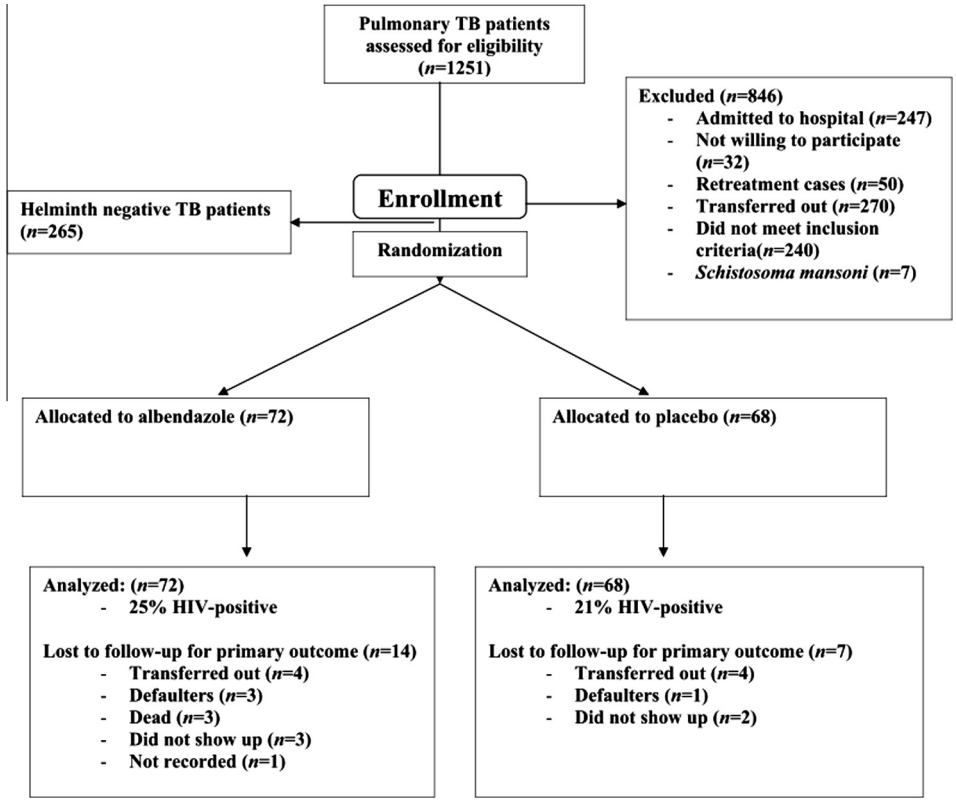

Fig. 1. Flow chart of the enrolment process for tuberculosis patients in the study trial conducted at three Directly Observed Treatment Short-Course Clinics at the Gondar University Teaching Hospital, Debark Hospital and Gondar Health Centre, Ethiopia.

in the levels of Tregs, IFN-γ, IL-5 or IL-10 at baseline (Table 1) or following sub-grouping for HIV.

3.3. Effects of albendazole treatment on primary and secondary outcomes in helminth-positive TB patients

Following deworming, there was a significant decrease in helminth infection in the albendazole treated group compared with placebo at week 12 (8% versus 39%, $P < 0.001$). Among the 8% (4/49) of patients in the albendazole group who remained helminth-positive, two patients had *Ascaris*, one had *Trichuris* and one had *Strongyloides*. No significant effect of albendazole treatment on the primary outcome (TB score change from week 0 to week 8) was found between the albendazole and placebo groups (5.6 ± 2.87 points, $n = 58$ versus 5.9 ± 2.5; $n = 61$, $P = 0.59$, Table 2). A pronounced decrease in eosinophil cells was observed at week 12 in albendazole treated TB patients compared with the placebo group (Δ Eosinophil cells: 379 ± 434 cells/mm^3, $n = 52$ versus 112 ± 411 cells/mm^3, $n = 51$, $P = 0.001$, Fig. 2). No differences were detected for other secondary outcomes (changes in CXR, smear conversion rate and IgE level) (Table 2). A non-significant increase in the absolute mean change of CD4+ T cells from baseline to 3 months was observed in patients given albendazole (absolute increase: +107 CD4+ T cells/mm^3 versus +42 CD4+ T cells/mm^3 in the placebo group; $P = 0.16$) (Table 3). This effect was present primarily in HIV-negative albendazole treated patients compared with the placebo group (+122 CD4+ T cells/mm^3 versus +42 CD4+ T cells/mm^3; $P = 0.17$).

3.4. Subgroup and exploratory analyses of the effects of albendazole treatment on TB patients

No significant difference was observed in the mortality rate at 6 months between the albendazole and placebo groups (5.9% versus 6.3%, $P = 0.91$). Furthermore, no significant differences were observed in the primary or secondary outcomes in a sub-group analysis of patients who had *Ascaris* infection at baseline (data not shown). With regard to the influence of HIV co-infection, a separate sub-group analysis of HIV-negative and HIV-positive patients did not reveal marked effects on primary and secondary outcomes other than the reduction in helminth load and eosinophil counts following albendazole treatment compared with placebo. As a separate analysis, the PRC after 12 weeks from baseline, for changes in eosinophil and CD4+ T cell levels and weight gain, was analysed as previously described (Borkow and Bentwich, 2004). This analysis showed that CD4+ T cell counts increased by 422 ± 2472%, $n = 44$ compared with placebo 32 ± 86%, $n = 36$, $P = 0.34$ and that eosinophil levels decreased in the albendazole group compared with placebo (−42 ± 70%, $n = 52$ versus 40 ± 330%, $n = 51$, $P = 0.08$). As an exploratory finding, TB patients treated with albendazole showed a trend towards a significant level of weight gain compared with the placebo group from week 0 to week 12 (11.2 ± 8.5 versus 8.2 ± 8.7 kg, $P = 0.08$) (Fig. 2).

3.5. Effects of immunological responses following albendazole treatment in helminth-positive TB patients compared with placebo

For assessment of immunological responses after 12 weeks (Tregs, IFN-γ, IL-5 and IL-10), a subset of 32 TB patients from the randomised cohort were analysed. The HIV co-infection rate for this subset was 50% (6/12) and 15% (3/20), for the albendazole and placebo groups, respectively ($P = 0.04$). In a multivariate analysis at week 12 after albendazole treatment, following adjustment for baseline IL-10 value, age, sex and HIV status, a significant decline in the level of IL-10 spot forming units (SFU) of PBMCs was observed in the albendazole group compared with placebo at week 12 (125 ± 190 versus 204 ± 168, $P = 0.017$). There were

Table 1
Baseline characteristics of randomised helminth-positive tuberculosis patients.

	Albendazole treated $n = 72$			Placebo $n = 68$			P
	Mean	S.D.	n	Mean	S.D.	n	
Age (years)	30.7	12.7	72	31	12.6	68	NS
Females (%)	65		72	57		68	NS
HIV-positive (%)	25		72	21		68	NS
CD4+ cells/mm^3	406	248	70	551	349	65	0.006
TB score (points)	8.03	2.5	72	7.75	2.7	68	0.53
Sputum SP (%)	68			64			NS
IgE (IU/L)	955	1456	65	771	1479	58	NS
Eosinophil, (cells/mm^3)	580	565	64	517	480	64	NS
CXR			42			34	
Normal (%)	7			6			NS
Mild (%)	21			21			NS
Moderate (%)	29			41			NS
Far advanced (%)	43			32			NS
Ascaris lumbricoides (%)	53		72	52		68	NS
Hookworm (%)	17		72	21		68	NS
Strongyloides stercoralis (%)	15		72	13		68	NS
Trichuris trichiura (%)	10		72	12		68	NS
Hymenolepis nana (%)	3		72	2		68	NS
Taenia spp. (%)	3		72	4		68	NS
Tregs (%/CD4)	2.47	1.65	7	2.14	1.52	18	NS
IFN-γ Unst (SFU)	92	86	15	85	70	22	NS
IFN-γ PPD (SFU)	214	131	15	210	101	22	NS
IL-5 Unst (SFU)	75	53	15	51	47	22	NS
IL-5 PPD (SFU)	134	104	15	145	93	22	NS
IL-10 Unst (SFU)	130	155	15	146	176	22	NS
IL-10 PPD (SFU)	181	195	15	195	178	22	NS

SP, sputum positive; CXR, chest X-ray; Tregs, regulatory T cells; Unst, unstimulated; PPD, purified protein derivative-stimulated; SFU, spot forming units/250,000 peripheral blood mononuclear cells; NS, not significant.

no significant differences in Tregs, or PPD-induced IFN-γ and IL-5 responses between albendazole and placebo groups at week 12 (Fig. 3). A separate analysis of the immunological variables for the HIV-negative TB patients in the two groups did not show any significant differences.

4. Discussion

To our knowledge, the present study is the first randomised clinical trial investigating the impact of deworming on the clinical outcome of TB patients with active infection and simultaneously evaluating the immunological responses during helminth-TB co-infection. Although we found no effect on clinical improvement measured as the change in TB score after 8 weeks, the results clearly show that asymptomatic helminth infection during active TB significantly affects host immunity, as the levels of eosinophils and IL-10 declined following albendazole treatment compared with the placebo group. Additionally, in the exploratory analysis there was a trend towards weight gain following deworming.

Table 2
Effect of deworming on helminth-positive patients with active tuberculosis.

Mean ± S.D.	Albendazole		Placebo		P[a]
	Total	n	Total	n	
Primary outcome					
ΔTB-score (W0-W8)	5.6 ± 2.87	58	5.9 ± 2.54	61	0.5
Secondary outcomes					
Smear conversion W8 (%)	87	54	93	55	0.5
Changes in CXR (W0-W12)		27		22	
Normal (%)	3.6		4.3		NS
Regression (%)	92.8		91.3		NS
Stable disease (%)	3.6		4.4		NS
Progression (%)	0		0		NS
ΔIgE KU/L (W0–W12)	181 ± 631	39	151 ± 608	41	NS
ΔEosinophil (W0–W12)	379 ± 434	52	112 ± 411	51	0.001
Helminth positive (%, (n))	8 (4)	49	39 (18)	46	0.001

Δ, Delta change; W, week; CXR, chest X-ray; NS, not significant.
[a] P value between helminth-positive tuberculosis patients in albendazole and placebo groups.

It has previously been shown that elevated IL-10 levels are associated with susceptibility to TB and mice lacking IL-10 were shown to clear BCG infection more efficiently (Turner et al., 2008). Mice over-expressing IL-10 were more susceptible to Mtb infection and had decreased numbers of activated T cells in the peripheral blood and lung tissues (Murray, 1999). IL-10 is primarily produced by Tregs and AAMs and it has been shown that Tregs are up-regulated during helminth infection (Resende Co et al., 2007). In a recent experimental study of helminth infection, AAMs had a decreased ability to control growth of Mtb (Potian et al., 2011) and AAMs are abundant during helminth co-infection in humans (Babu et al., 2009). Thus, one plausible explanation for the decrease in IL-10 observed in the current study is an effect of deworming on the levels and responses of Tregs and AAMs. In support of our findings for the decrease in IL-10, a clinical trial on the effect of albendazole in HIV patients without TB also found a reduction in IL-10 in plasma compared with placebo (Blish et al., 2010). Thus, the elevated IL-10 cytokine levels in TB-helminth co-infected patients, together with a significant decline during albendazole treatment, supports the immunomodulatory effect of helminth infection, and treatment with albendazole might improve cell mediated immune responses against intracellular infections such as TB.

A highly significant decline in peripheral eosinophil counts from baseline was observed in patients treated with albendazole compared with the placebo group. Although it is clear that there is a Th2-linked increase in eosinophils induced by IL-5 following helminth infection, the relative importance of eosinophils in the host resistance against human helminth infection is unclear (Anthony et al., 2007). Eosinophil infiltration to local sites of helminth infection has been shown in humans harbouring intestinal helminths such as *Trichuris trichiura* (Kaur et al., 2002). The reduction in eosinophils following albendazole treatment of helminth infection has been previously observed in several studies (Maxwell et al.,

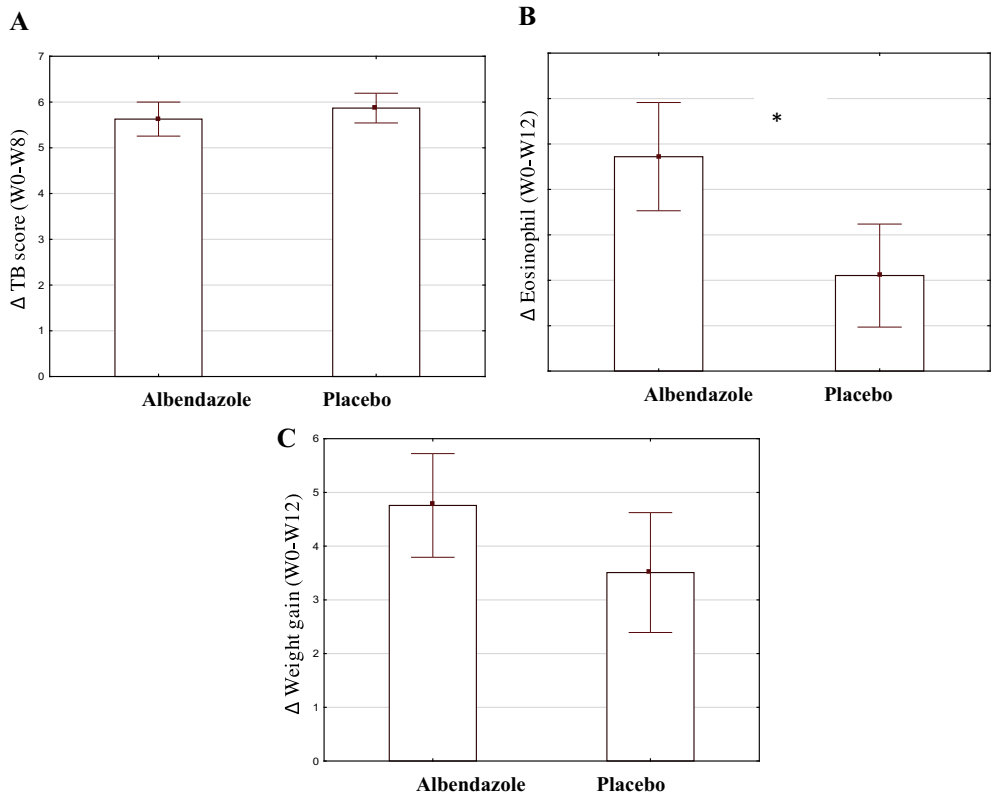

Fig. 2. Participants were randomly assigned to groups and treated with either albendazole or a placebo (400 mg per day for 3 days) at baseline and followed for 12 weeks. The graphs show the delta changes (Δ) of (A) tuberculosis score, $P = 0.5$, (B) eosinophils, $P = 0.001$ and (C) weight gain, $P = 0.08$, between two measurements before (week (W) 0) and after deworming (W12). Each bar represents the percentage of relative change (mean ± S.E.M.) for each group. *Indicates the level of significance.

Table 3
Effect of deworming on immunological markers in helminth-positive patients with active tuberculosis. Data are presented as mean ± S.D. for week 12 levels of lymphocyte subsets and cytokines analysed by Enzyme-Linked ImmunoSpot.

	Albendazole					Placebo					P^a	P^b
	Total	HIV+	*n*	HIV-	*n*	Total	HIV+	*n*	HIV-	*n*		
CD4+ cells	522 ± 273	232 ± 188	12	627 ± 219	33	565 ± 261	219 ± 113	8	657 ± 204	30	0.57	0.46
ΔCD4+ cells	107 ± 214					42 ± 187						0.15
Tregs %	1.97 ± 1.59	2.6 ± 1.32	5	1.53 ± 1.71	7	1.89 ± 1.53	0.76 ± 0.43	2	2.04 ± 1.57	15	0.5	0.88
IFN-γ Unst	83 ± 76	73 ± 54	6	92 ± 98	6	81 ± 74	88 ± 81	3	80 ± 75	17	0.79	0.94
IFN-γ PPD	209 ± 118	201 ± 105	6	216 ± 139	6	181 ± 112	127 ± 64	3	190 ± 117	17	0.69	0.51
IL-5 Unst	59 ± 41	72 ± 39	6	47 ± 43	6	49 ± 34	40 ± 14	3	51 ± 37	17	0.8	0.47
IL-5 PPD	166 ± 97	190 ± 99	6	142 ± 98	6	175 ± 92	151 ± 57	3	179 ± 97	17	0.5	0.8
IL-10 Unst	33 ± 42	55 ± 53	6	12 ± 7	6	61 ± 77	30 ± 42	3	67 ± 81	17	0.11[c]	0.25
IL-10 PPD	125 ± 190	129 ± 142	6	121 ± 243	6	204 ± 168	193 ± 174	3	206 ± 172	17	0.45	0.23

Δ, Delta change; Tregs, regulatory T cells; Unst, unstimulated; PPD, purified protein derivative-stimulated.
[a] HIV-negative tuberculosis patients in the albendazole group compared with the placebo group (Student's *t*-test).
[b] Total tuberculosis patients in the albendazole group compared with the placebo group (Student's *t*-test).
[c] $P = 0.017$ in a multiple regression model including age, sex and HIV status.

1987; Wright et al., 2009) but not in TB patients with asymptomatic infection. We previously reported a significant correlation between eosinophilia and asymptomatic helminth infection in TB patients (Abate et al., 2012). Thus, the observed reduction in eosinophils could be regarded as an indirect measurement of reduced worm load and Th2 response. Taken together, the reduction in eosinophils and IL-10 strongly suggests that asymptomatic helminth infection during TB induces a Th2 type immune response which could be reversed by albendazole.

We chose TB score as a primary outcome for evaluation of clinical improvement. The TB score is composed of clinical signs and symptoms and has been used previously in clinical trials (Wejse et al., 2009). Analysis of the change in TB score did not show any statistical effect of deworming on the clinical outcome for TB patients. One reason might be that the effect of the TB treatment has a much greater impact on the TB score than the effect of deworming as the TB score was already greatly reduced at week 2 when the albendazole was introduced. Thus, even though this clinical scoring system was validated in highly endemic TB areas (Wejse et al., 2008; Janols et al., 2012) for patient monitoring in clinical studies, the TB score might not be sensitive enough to detect subtle clinical changes such as those caused by helminth infection. A clear limitation in that regard is also that the final sample size for the study was not reached and that the effects of deworming during TB infection, including sub-grouping for HIV status, could be masked by the major clinical signs of TB and HIV.

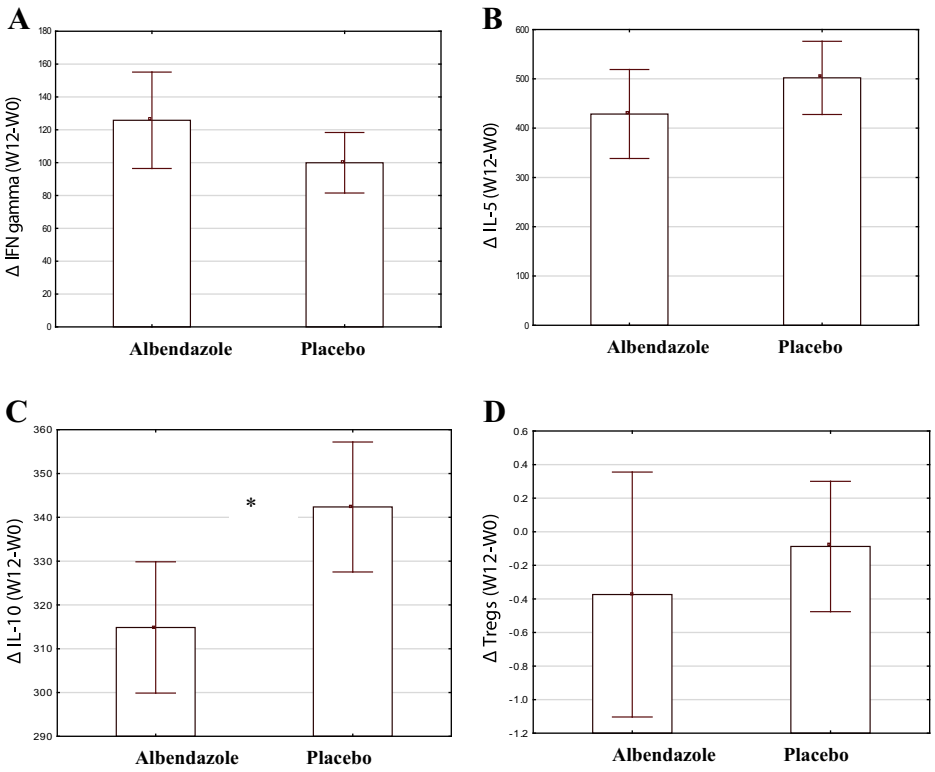

Fig. 3. Following random assignment to groups, helminth co-infected tuberculosis patients received albendazole or a placebo (400 mg per day for 3 days) and were assessed for changes (week (W)12-W0) in the level of purified protein derivative-stimulated (A) IFN-γ SFU, (B) IL-5 SFU, (C) IL-10 SFU and (D) percentage of regulatory T cells (Tregs) at W0 and 12 weeks after albendazole ($n = 11$) or placebo treatment ($n = 16$) of tuberculosis patients co-infected with helminths. No significant changes were observed except for a significant decrease (*) in IL-10 when correcting for age, sex and HIV in a multivariate regression analysis ($P = 0.017$). The error bars show mean ± S.E.M. (SFU, spot forming unit/250,000 peripheral blood mononuclear cells).

Other than eosinophils and IL-10, no significant changes were observed in secondary outcome measurements including changes in the immunological response after month 3 in Tregs, IFN-γ and IL-5 levels (Fig. 3). This is consistent with a previous study evaluating the effect of deworming in HIV patients which did not show any significant changes in plasma cytokine responses (IFN-γ, IL-2, IL-4, IL-5 and IL-13) other than IL-10 (Blish et al., 2010). Those patients were recruited in a randomised clinical trial on albendazole treatment during HIV infection without TB, where there was a significant increase in mean CD4+ T cell counts at follow-up in the sub-group of patients infected with *Ascaris* compared with the placebo group (+109 CD4+ cells/mm^3), and a trend for lower HIV-1 RNA levels (Kaur et al., 2002; Turner et al., 2008). In the current study, we similarly observed a non-significant increase in CD4+ T cells but primarily among the HIV-negative TB patients. In the sub-group analysis for *Ascaris* infection there was no effect of deworming in any of the primary or secondary outcomes, although the small sample size compromised this analysis (data not shown).

We believe this is the first controlled clinical trial on effects of deworming in TB patients including immunological readouts but we acknowledge that it has limitations. The most important one is that the study is not adequately powered to rule out a clinical effect of albendazole treatment. In that regard, it should be noted that the sub-group analyses should be interpreted with caution. A low enrolment rate and lack of resources to continue the study for more than 3 years limited recruitment. In the immunological analyses, the skewed distribution of the HIV co-infected patients as well as the absence of stimulation with helminth antigens limit the possibility to make firm conclusions about effects on the host response against TB. The optimal time to follow up immunological changes after deworming is not known and 12 weeks may have been too early, which in turn could have underestimated potential differences. Nevertheless, we still detected significant changes in eosinophilia and levels of IL-10 at the 12 week evaluation.

In conclusion, albendazole treatment did not affect the clinical outcome of helminth co-infected TB patients as evaluated by the TB score after 2 months. Deworming of helminth co-infected TB patients was effective and induced a significant decline in peripheral eosinophils and IL-10. The reduction in IL-10 observed with albendazole treatment in this study has a potential impact on the course of TB since IL-10 is a potent immunomodulatory cytokine that suppresses the induction of a specific, Th1-directed immune response to TB. Larger multi-centre studies are warranted to further investigate the clinical and immunological effects of deworming on the outcome of pulmonary TB.

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References

Abate, E., Belayneh, M., Gelaw, A., Idh, J., Getachew, A., Alemu, S., Diro, E., Fikre, N., Britton, S., Elias, D., Aseffa, A., Stendahl, O., Schön, T., 2012. The impact of asymptomatic helminth co-infection in patients with newly diagnosed tuberculosis in north-west Ethiopia. PLoS One, e42901.

American Thoracic Society, 1961. National Tuberculosis Association of the USA: Diagnostic standards and Classification of Tuberculosis. National Tuberculosis Association, New York.

Anthony, R.M., Rutitzky, L.I., Urban, J.F., Stadecker, M.J., Gause, W.C., 2007. Protective immune mechanisms in helminth infection. Nat. Rev. Immunol. 7, 975–987.

Babu, S., Kumaraswami, V., Nutman, T.B., 2009. Alternatively activated and immunoregulatory monocytes in human filarial infections. J. Infect. Dis. 199, 1827–1837.

Bentwich, Z., Kalinkovich, A., Weisman, Z., Borkow, G., Beyers, N., Beyers, A.D., 1999. Can eradication of helminthic infections change the face of AIDS and tuberculosis? Immunol. Today 20, 485–487.

Blish, C.A., Sangaré, L., Herrin, B.R., Richardson, B.A., John-Stewart, G., Walson, J.L., 2010. Changes in plasma cytokines after treatment of *Ascaris lumbricoides* infection in individuals with HIV-1 infection. J. Infect. Dis. 201, 1816–1821.

Borkow, G., Bentwich, Z., 2004. Chronic immune activation associated with chronic helminthic and human immunodeficiency virus infections: role of hyporesponsiveness and anergy. Clin. Microbiol. 17, 1012–1030.

Borkow, G., Weisman, Z., Leng, Q., Stein, M., Kalinkovich, A., Wolday, D., Bentwich, Z., 2001. Helminths, human immunodeficiency virus and tuberculosis. Scand. J. Infect. Dis. 33, 568–571.

Brighenti, S., Andersson, J., 2012. Local immune responses in human tuberculosis: learning from the site of infection. J. Infect. Dis. 2, S316–S324.

Elias, D., Akuffo, H., Britton, S., 2006. Helminthes could influence the outcome of vaccines against TB in the tropics. Parasite Immunol. 28, 507–513.

Elias, D., Akuffo, H., Pawlowski, A., Haile, M., Schön, T., Britton, S., 2005. *Schistosoma mansoni* infection reduces the protective efficacy of BCG vaccination against virulent *Mycobacterium tuberculosis*. Vaccine 23, 1326–1334.

Elias, D., Britton, S., Aseffa, A., Engers, H., Akuffo, H., 2008. Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-beta production. Vaccine 26, 3897–3902.

Federal Ministry of Health (FMOH), HIV/AIDS Prevention and Control Office (HAPCO), Drug Administration and Control Authority (DACA), 2003. Guideline in the Use of Antiretrovirals in Ethiopia. MOH/HAPCO/DACA, Addis Ababa, Ethiopia.

Gaze, S., McSorley, H.J., Daveson, J., Jones, D., Bethony, J.M., Oliveira, L.M., Speare, R., McCarthy, J.S., Engwerda, C.R., Croese, J., Loukas, A., 2012. Characterizing the mucosal and systemic immune responses to experimental human hookworm infection. PLoS Pathog. 8, e1002520.

Janols, H., Abate, E., Idh, J., Senbeto, M., Britton, S., Alemu, S., Aseffa, A., Stendahl, O., Schön, T., 2012. Early treatment response evaluated by a clinical scoring system correlates with the prognosis of pulmonary tuberculosis patients in Ethiopia: a prospective follow-up study. Scand. J. Infect. Dis. 44, 828–834.

Kaur, G., Raj, S.M., Naing, N.N., 2002. Trichuriasis: localized inflammatory responses in the colon. Southeast Asian J. Trop. Med. Public Health 33, 224–228.

Lyke, K.E., Burges, R.B., Cissoko, Y., Sangare, L., Kone, A., Dao, M., Diarra, I., Fernández-Vina, M.A., Plowe, C.V., Doumbo, O.K., Sztein, M.B., 2005. HLA-A2 super type restricted cell-mediated immunity by peripheral blood mononuclear cells derived from Malian children with severe or uncomplicated *Plasmodium falciparum* malaria and healthy controls. Infect. Immun. 73, 5799–5808.

Marin, N.D., París, S.C., Vélez, V.M., Rojas, C.A., Rojas, M., García, L.F., 2010. Regulatory T cell frequency and modulation of IFN-gamma and IL-17 in active and latent tuberculosis. Tuberculosis 90, 252–261.

Maxwell, C., Hussain, R., Nutman, T.B., Poindexter, R.W., Little, M.D., Schad, G.A., Ottesen, E.A., 1987. The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. Am. J. Trop. Med. Hyg. 37, 126–134.

Murray, P.J., 1999. Defining the requirements for immunological control of mycobacterial infections. Trends Microbiol. 7, 366–372.

Potian, J.A., Rafi, W., Bhatt, K., McBride, A., Gause, W.C., Salgame, P., 2011. Preexisting helminth infection induces inhibition of innate pulmonary anti-tuberculosis defense by engaging the IL-4 receptor pathway. J. Exp. Med. 208, 1863–1874.

Redford, P.S., Murray, P.J., O'Garra, A., 2011. The role of IL-10 in immune regulation during *M. tuberculosis* infection. Mucosal Immunol. 4, 261–270.

Resende Co, T., Hirsch, C.S., Toossi, Z., Dietze, R., Ribeiro-Rodrigues, R., 2007. Intestinal helminth co-infection has a negative impact on both anti-*Mycobacterium tuberculosis* immunity and clinical response to tuberculosis therapy. Clin. Exp. Immunol. 147, 45–52.

Rook, G.A., 2007. Th2 cytokines in susceptibility to tuberculosis. Curr. Mol. Med. 7, 327–337.

Salgame, P., Yap, G.S., Gause, W.C., 2013. Effect of helminth-induced immunity on infections with microbial pathogens. Nat. Immunol. 14, 1118–1126.

Sleigh, A., Hoff, R., Mott, K., Barreto, M., de Paiva, T.M., Pedrosa Jde, S., Sherlock, I., 1982. Comparison of filtration staining (Bell) and thick smear (Kato) for the detection of quantitation of *Schistosoma mansoni* eggs in faeces. Trans. R. Soc. Trop. Med. Hyg. 76, 403–406.

Tameris, M.D., Hatherill, M., Landry, B.S., Scriba, T.J., Snowden, M.A., Lockhart, S., Shea, J.E., McClain, J.B., Hussey, G.D., Hanekom, W.A., Mahomed, H., McShane, H., 2013. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet 381, 1021–1028.

Tristão-Sá, R., Ribeiro-Rodrigues, R., Johnson, L.T., Pereira, F.E., Dietze, R., 2002. Intestinal nematodes and pulmonary tuberculosis. Rev. Soc. Bras. Med. Trop. 35, 533–535.

Turner, J.D., Jackson, J.A., Faulkner, H., Behnke, J., Else, K.J., Kamgno, J., Boussinesq, M., Bradley, J.E., 2008. Intensity of intestinal infection with multiple worm species is related to regulatory cytokine output and immune hyporesponsiveness. J. Infect. Dis. 197, 1204–1212.

Wejse, C., Gomes, V.F., Rabna, P., Gustafson, P., Aaby, P., Lisse, I.M., Andersen, P.L., Glerup, H., Sodemann, M., 2009. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am. J. Respir. Crit. Care Med. 179, 843–850.

Wejse, C., Gustafson, P., Nielsen, J., Gomes, V.F., Aaby, P., Andersen, P.L., Sodemann, M., 2008. TB score: signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. Scand. J. Infect. Dis. 40, 111–120.

World Health Organization Global Tuberculosis Report, 2012. Geneva, Switzerland. Available http://www.who.int/tb/publications/global_report/gtbr12_main.pdf. ISBN 978 92 4 156450 2.

Wright, V.J., Ame, S.M., Haji, H.S., Weir, R.E., Goodman, D., Pritchard, D.I., Mohamed, M.R., Haji, H.J., Tielsch, J.M., Stoltzfus, R.J., Bickle, Q.D., 2009. Early exposure of infants to GI nematodes induces Th2 dominant immune responses which are unaffected by periodic antihelminthic treatment. PLoS Negl. Trop. Dis. 3, e433.